MADROÑO, Vol. 58, No. 4, pp. 249–255, 2011

MOLECULAR PHYLOGENETICS OF *GARRYA* (GARRYACEAE)

DYLAN O. BURGE[1]
Duke University Department of Biology, Box 90338, Durham, NC 27708
dylan.o.burge@gmail.com

ABSTRACT

Garrya (Garryaceae) comprises 15 species of shrubs and small trees restricted to the Americas. *Garrya* is taxonomically divided into two subgenera, *Garrya* and *Fadyenia*, which differ in morphology, secondary chemistry, and geographic distribution. The present work uses nuclear ribosomal DNA (ITS) sequence data from 11 *Garrya* species to elucidate phylogenetic relationships within the genus and test the monophyly of the subgenera. Results strongly support subgenus *Fadyenia* as monophyletic, while monophyly of subgenus *Garrya* is supported only by maximum parsimony analyses. ITS data do not provide evidence for genetic admixture between the two subgenera of *Garrya* in spite of broad geographic overlap.

Key Words: *Aucuba*, California, *Eucommia*, *Fadyenia*, *Garrya*, Garryaceae, ITS, Mexico.

Garrya Douglas ex Lindl. contains 15 species endemic to North America, Central America, and the Caribbean (Dahling 1978; Nesom unpublished; Table 1; Fig. 1). *Garrya* species are dioecious shrubs and small trees with decussate, evergreen leaves and pendulous, catkin-like inflorescences. The plants are probably wind pollinated (Hallock 1930; Dahling 1978; Liston 2003). *Garrya* is found in a diversity of habitats over its broad geographic range, from cloud forest to maritime chaparral, but is typically a component of shrublands (e.g., chaparral) or forests (Dahling 1978).

Molecular phylogenetic studies consistently resolve *Garrya* as sister to the east Asian shrub genus *Aucuba* (Soltis et al. 2000; Bremer et al. 2002), which together comprise Garryaceae (APG 2003). These results confirm a close relationship that has long been hypothesized on the basis of morphology and chemistry (reviewed in Liston 2003). Molecular phylogenetic studies also support a close relationship between Garryaceae and the monotypic east Asian tree *Eucommia* (Eucommiaceae), which together comprise the euasterid order Garryales (APG 2003).

Garrya is divided into two subgenera, *Garrya* (6 spp.) and *Fadyenia* (9 spp.), which differ in geographic distribution, inflorescence morphology, and secondary chemistry (Dahling 1978; Table 2). Subgenus *Fadyenia* has its center of diversity in Mexico, while subgenus *Garrya* reaches peak diversity in the western U.S. The geographic distribution of the subgenera overlaps in the southwestern U.S. and Mexico (Dahling 1978). Research presented here aims to elucidate phylogenetic relationships in *Garrya* and relate this to the taxonomy, distribution, and biology of the species. Specifically, I test the hypothesis that the *Garrya* subgenera are monophyletic, with separate histories of diversification in different geographic regions of the Americas.

MATERIALS AND METHODS

Genetic Sampling

Sampling of *Garrya* populations was designed to represent the geographic range of the genus with an emphasis on California, the southwestern U.S., and Mexico (Table 3; Appendix 1; Fig. 1). One sample of *Aucuba* was obtained from a garden planting in California. DNA from 22 *Garrya* individuals was studied, representing 11 of the 15 species currently recognized (Dahling 1978; Nesom unpublished). For the species occurring in the U.S., voucher specimens were identified according to Nesom (unpublished); for all exclusively Latin American taxa, identifications were according to Dahling (1978). Voucher specimens are deposited at DUKE (Appendix 1).

Molecular Methods

Genomic DNA was extracted from silica-dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Polymerase chain reactions were performed using Qiagen *Taq* DNA Polymerase. Amplification was performed using an initial incubation at 94°C for 10 min and 30 cycles of three-step PCR (1 min at 94°C, 30 sec at 55°C, and 2 min at 72°C), followed by final extension at 72°C for 7 min. I amplified the nuclear ribosomal ITS region (ITS 1, 5.8S, and ITS2) using the primers ITS4 (White et al. 1990) and ITSA (Blattner 1999). I amplified the *trn*L-F plastid region, comprising the *trn*L intron and the

[1] Present address: National Herbarium of New South Wales, Royal Botanic Gardens, Sydney, Mrs Macquaries Road, New South Wales, 2011, Australia.

TABLE 1. *GARRYA* SPECIES AND SAMPLING. **Sampling**: number of populations sampled for phylogenetic analysis (Table 3); **Distribution**: geographic distribution of the species (SW U.S.: southwestern United States); **CFP**: indicates whether species occurs in the California Floristic Province; **Flower:** range of known flowering times for species (Dahling 1978). Ω subspecies are recognized by Dahling (1978) and/or Nesom (unpublished): *G. ovata*: 3 subspecies; *G. laurifolia*: 4.

Species	Sampling	Distribution	CFP	Flower
Subgenus *Fadyenia*				
G. fadyena Hook.	0	Greater Antilles		Dec-Feb
G. glaberrima Wangerin	3	E Mexico		Mar-May
G. grisea Wiggins	1	Mexico (Baja California)	X	Feb-Apr
G. laurifolia Benth.Ω	2	Mexico and Central America		Dec-Apr
G. lindheimeri Torr.	1	SW U.S., Mexico		Mar-May
G. longifolia Rose	0	Mexico		Jan-Mar
G. ovata Benth.Ω	1	SW U.S. and Mexico		Mar-Apr
G. salicifolia Eastw.	0	Mexico (Baja California Sur)		Aug-Dec
G. wrightii Torr.	3	SW U.S. and Mexico		Apr-Aug
Subgenus *Garrya*				
G. buxifolia A. Gray	1	U.S. (CA and OR)	X	Feb-Apr
G. corvorum Standl. & Steyerm.	0	Guatemala		Dec-Jan
G. elliptica Douglas ex Lindl.	2	U.S. (CA and OR)	X	Dec-Feb
G. flavescens S. Watson	3	U.S. (AZ, CA, NV NM, UT) and Mexico	X	Feb-Apr
G. fremontii Torr.	3	U.S. (CA, OR, WA)	X	Jan-Apr
G. veatchii Kellogg	2	U.S. (CA) and Mexico (Baja California)	X	Jan-May

*trn*L-F intergenic spacer, using primers c and f of Taberlet et al. (1991). Excess primer and dNTPs were removed using exonuclease I (New England Biolabs, Ipswich, MA [NEB]; 0.2 units/μl PCR product) and antarctic phosphatase (NEB; 1.0 unit/μl PCR product) incubated for 15 min at 37°C followed by 15 min at 80°C. For sequencing, Big Dye chemistry (Applied Biosystems, Foster City, CA) was utilized according to the manufacturer's instructions. Sequences were determined on an Applied Biosystems 3100 Genetic Analyzer at the Duke University Institute for Genome Science and Policy Sequencing and Genetic Analysis Facility.

Sequences and Alignment

A total of 23 ITS and 12 *trn*L-F sequences were generated for the present study. A preliminary alignment of *trn*L-F revealed that the nine sequenced *Garrya* species shared a nearly identical sequence; there was just a single nucleotide substitution difference among the species, a change unique to *G. grisea* Wiggins (D.O. Burge 778; Table 3). In addition, two insertion/deletion events were present within *Garrya*, 1) a 1 bp length difference within the *trn*L intron, where a poly-T region was one bp longer in two accessions of *G. elliptica* Douglas ex Lindl. (D.O. Burge 382 & 386; Table 3) than in remaining *Garrya*, and 2) a 2 bp length difference in the *trn*L-F intergenic spacer, where a poly-T region was two bp longer in members of subgenus *Fadyenia* relative to members of subgenus *Garrya*. Because of this low level of variation, *trn*L-F was not sequenced in additional plants, and was abandoned in favor of ITS for subsequent alignment and tree building.

The 23 new ITS sequences (22 *Garrya* and 1 *Aucuba*) were supplemented with an ITS sequence for *Eucommia ulmoides* Oliv. from GenBank (Table 3). All DNA sequences were assembled and edited using Sequencher 4.1 (Gene Codes Corporation). Edited sequences were deposited in GenBank (*trn*L-F: JN234721-32; ITS: Table 3). ITS sequences were aligned using MUSCLE (Edgar 2004) under default settings. Due to ambiguity, a 22 bp region of ITS1 was excluded from all subsequent analyses. The alignment was deposited in TreeBase (Study 11755).

Phylogenetic Analysis

Trees were reconstructed using Bayesian, maximum likelihood (ML), and maximum parsimony (MP) techniques. Trees were rooted using *Eucommia ulmoides* (APG 2003). Bayesian phylogenetic analyses were conducted using the best-fit model of evolution from AIC output of the program MrModeltest (GTR + G; Nylander 2004). Sampling of trees was performed using the program MrBayes 3.0 (Ronquist and Huelsenbeck 2003). Three separate runs of 1×10^6 MCMC generations were performed using one heated and three cold chains, sampling every 1000 generations. Independent chains were inspected for convergence (standard deviation of

FIG. 1. *Garrya* distribution and sampling. Distribution of *Garrya* indicated by dark gray shading (data from participants of the Consortium of California Herbaria, 2011). Sampling locations indicated by white circles (Table 3).

split frequencies nearing 0.001). Log-likelihood for the sampled tree was plotted and examined in Microsoft Excel to assess convergence and determine an appropriate burn-in period (Ronquist and Huelsenbeck 2003). A total of 1×10^5 generations (100 trees) were eliminated as burn-in, leaving 9×10^5 generations (950 trees) of explored tree space for computing branch lengths and posterior probabilities (PP) of clades. Consensus phylograms were built for each of the three independent runs using MrBayes (Ronquist and Huelsenbeck 2003). Following inspection to verify similarity of the results, trees from all three runs were combined in a consensus phylogram. Maximum likelihood tree building was performed in GARLI v 1.0

TABLE 2. MORPHOLOGICAL AND DISTRIBUTIONAL COMPARISON BETWEEN THE TWO SUBGENERA OF *GARRYA*.

Character	Description	
	Subgenus *Garrya*	Subgenus *Fadyenia*
♀ inflorescence	Compact, pendulous, unbranched	Loose, erect, branched
Ovary appendage	Small, epigynous	Large, foliaceous, partially adnate
Paired floral bracts		
Flowers per pair	Three	One
Fusion of pair	Basally connate, forming a cup	Distinct to partially adnate basally
Size in ♀	Reduced in size, not leaf-like	At least proximal large and leaf-like
Geographic distribution	Western U.S. and northern Mexico	Western U.S., Mexico, Central America, and Caribbean

Table 3. Collection Number and Provenance for Voucher Specimen (Appendix 1) and GenBank Accession Numbers for ITS Sequences. All vouchers deposited at DUKE.

Taxon	Collection number and provenance	GenBank ITS
Aucuba japonica Thunb.	D.O. Burge 363, Butte Co., CA	JN234733
Eucommia ulmoides A. Gray	--	AY650006
Garrya, subgenus *Fadyenia*		
G. glaberrima Wangerin	D.O. Burge 1025, Hidalgo, Mexico	JN234743
	D.O. Burge 1216, Nuevo Leon, Mexico	JN234744
	D.O. Burge 1225, Tamaulipas, Mexico	JN234745
G. grisea Wiggins	D.O. Burge 778, Baja California, Mexico	JN234746
G. laurifolia Benth.	D.O. Burge 1218, Nuevo Leon, Mexico	JN234747
	D.O. Burge 1252, Chihuahua, Mexico	JN234748
G. lindheimeri Torr.	D.O. Burge 750, Travis Co., TX	JN234749
G. ovata Benth.	D.O. Burge 1221, Nuevo Leon, Mexico	JN234750
G. wrightii Torr.	D.O. Burge 934, Pima Co., AZ	JN234753
	D.O. Burge 1239, Durango, Mexico	JN234754
	D.O. Burge 1253, Chihuahua, Mexico	JN234755
Garrya, subgenus *Garrya*		
G. buxifolia A. Gray	D.O. Burge 1160, Josephine Co., OR	JN234734
G. elliptica Douglas ex Lindl.	D.O. Burge 382, Monterey Co., CA	JN234735
	D.O. Burge 386, Marin Co., CA	JN234736
G. flavescens S. Watson	D.O. Burge 370, Kern Co., CA	JN234737
	D.O. Burge 419, Yavapai Co., AZ	JN234738
	D.O. Burge 1036, Baja California, Mexico	JN234739
G. fremontii Torr.	D.O. Burge 353, Butte Co., CA	JN234740
	D.O. Burge 362, Humboldt Co., CA	JN234741
	D.O. Burge 1148, Tuolumne Co., CA	JN234742
G. veatchii Kellogg	D.O. Burge 378, San Luis Obispo Co., CA	JN234751
	D.O. Burge 1041, Baja California, Mexico	JN234752

(Zwickl 2006). Two search replicates of 1×10^6 generations were performed in a single execution with a random starting tree. Other parameters were kept at default values. Statistical support was inferred with 100 replicates of bootstrap reweighting (Felsenstein 1985) using 5×10^5 generations per replicate. The majority rule consensus tree was calculated using the 100 best bootstrap trees. Maximum parsimony phylogenetic analysis was carried out using PAUP* v 4.0 (Swofford 2000). Heuristic searches used 1000 random sequence addition replicates and tree bisection-reconnection branch swapping. Nonparametric bootstrap analysis (Felsenstein 1985) was conducted using 100 pseudoreplicates and heuristic settings with 10 random sequence addition replicates. In all MP analyses, gaps introduced by the alignment process were treated as missing data.

Results

DNA Sequences

The ITS region for *A. japonica* Thunb. was 605 bp in length. In *Garrya* this region varied from 622 to 624 bp; in all members of subgenus *Garrya* the region was 624 bp long while in subgenus *Fadyenia* it varied from 622 (*G. grisea*, D.O. Burge 778; Table 3) to 623 bp. The ITS alignment (TreeBase Study 11755) contained 696 characters, 22 of which were excluded (see above). Of the 674 included characters, 189 were variable and 48 were parsimony informative.

Phylogeny

Bayesian, ML, and MP analyses provided similar topologies and levels of support (Fig. 2; TreeBase Study 11755). Maximum parsimony analysis resulted in 147 equally parsimonious trees (length = 217, CI = 0.97, RI = 0.97). A total of eight nodes are found in the strict consensus of these trees (Fig. 2). Overall, *Garrya* is strongly monophyletic (Bayesian PP 0.99; MP bootstrap 100%; ML boostrap 95%; Fig. 2); subgenus *Fadyenia* is also strongly supported as monophyletic (Bayesian PP 1.0; MP bootstrap 100%; ML boostrap 100%), with several moderately-supported groupings within it. Though subgenus *Garrya* is monophyletic in the strict consensus cladogram from MP analysis, and receives 89% MP bootstrap support, this group is not supported in Bayesian or ML analyses. In addition, a grouping of *Garrya ovata* Benth. with *Garrya lindheimeri* Torr. is strongly supported (Bayesian PP 1.0; MP bootstrap 86%; ML bootstrap 84%), as in a clade containing all sampled populations of *Garrya glaberrima* Wangerin and one *Garrya laurifolia* Benth. (Bayesian PP 0.99; MP bootstrap 93%; ML bootstrap 90%). However, none of the seven *Garrya* species

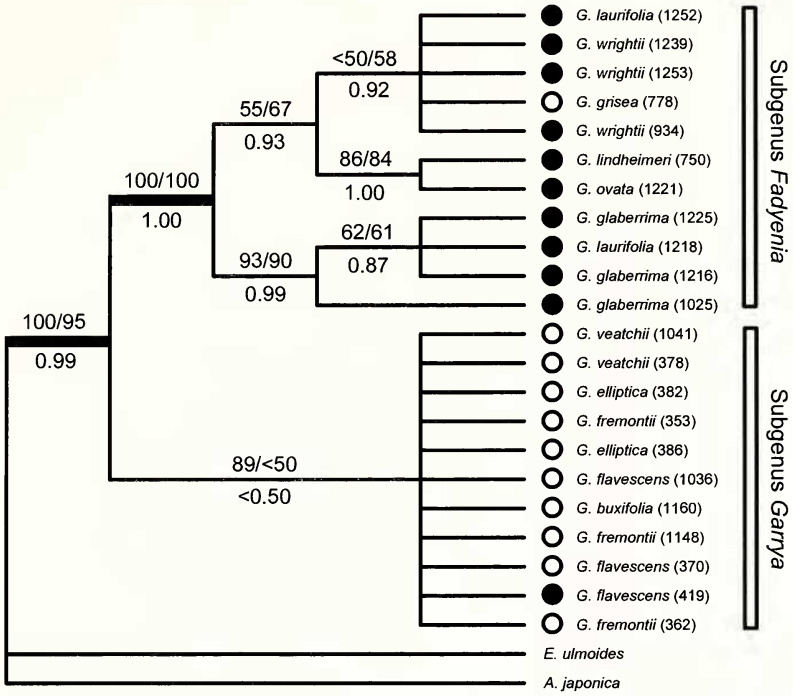

FIG. 2. Strict consensus of 147 equally parsimonious trees recovered in maximum parsimony (MP) phylogenetic analysis, with support values from 100 MP bootstrap replicates above branches, at left. Tree is rooted using *E. ulmoides*. Support from maximum likelihood bootstrap (above branches, at right) and Bayesian analysis (below branches) mapped on tree. Species names followed by D.O. Burge collection numbers. Open circles indicate collections obtained from within the California Floristic Province (CFP); dark circles are from outside of the CFP.

represented by more than one sampled plant are recovered as monophyletic (Fig. 2).

DISCUSSION

Phylogenetic Relationships

ITS trees strongly support subgenus *Fadyenia*, as circumscribed by Dahling (1978; Fig. 2). The Phylogenetic isolation of subgenus *Fadyenia* is supported by morphology, secondary chemistry (Dahling 1978), and geographic distribution (Table 2). By contrast, the monophyly of subgenus *Garrya* is strongly supported only by maximum parsimony trees (Fig. 2). The lack of support for subgenus *Garrya* that is seen in maximum likelihood and Bayesian analyses may represent an artifact of analysis due to the small size of the ITS dataset. Future studies should utilize additional genes from both the chloroplast and nuclear genomes.

The strong relationship between *G. ovata* and *G. lindheimeri* indicated by ITS is supported by the similar morphology of these species (Nesom unpublished). Indeed, the similarities are so great that *G. lindheimeri* was treated as part of *G. ovata*, at the subspecies rank, by Dahling (1978). Nevertheless, the species are ecologically distinct over most of their geographic range, and remain morphologically distinct in the parts of northern Mexico where they occur sympatrically, though hybrids may occasionally form (Nesom unpublished).

It is also noteworthy that no individual *Garrya* species is monophyletic (Fig. 2). One potential exception is *G. glaberrima*; the three included individuals of this species group together relatively strongly with a single individual of *G. laurifolia* (Fig. 2). The strong divergence of *G. glaberrima* from remaining members of subgenus *Fadyenia* is supported by the unusual morphology and phytochemistry of the species (Dahling 1978); presence of one individual of *G. laurifolia* (D.O. Burge 1281) in this group might be explained by geneflow, as this individual was collected in an area where *G. glaberrima* occurs (D.O. Burge 1216; Table 3; Figs. 1 and 2). The overall lack of monophyly for individual species of *Garrya* is noteworthy as it is consistent with the action of incomplete lineage sorting (Maddison and Knowles 2006) due to shallow genetic divergence among species, possibly exacerbated by geneflow. Hybrids are not frequently observed in the wild (Dahling 1978; D.O. Burge, personal observation; but see Munz and Keck 1968), and the extent of geneflow among species of *Garrya* has never been directly studied. Thus, incomplete lineage sorting stands as the most probable

explanation for the general lack of phylogenetic cohesion among populations of individual species. Nevertheless, the present study does not include all species, and is based on a small sample of populations; analysis of additional species and populations might reveal greater phylogenetic affinity among populations of individual species. In addition, the present study is based on a very small sample of DNA sequence data; additional data, ideally from both the chloroplast and nuclear genomes, might provide greater phylogenetic support for individual species.

Diversification of *Garrya* in the Americas

Subgenus *Fadyenia* represents a lineage that has diversified in the mountainous regions of the southwestern U.S., Mexico, Central America, and the Greater Antilles (Fig. 1). If subgenus *Garrya* is monophyletic, as suggested by some phylogenetic analyses (Fig. 2), the group would represent a diversification that is focused in the California Floristic Province (CFP) of western North America (Table 1, Fig. 2). In spite of the wide geographic overlap of these two groups in the southwestern U.S. and Mexico, which should present opportunities for interbreeding, molecular phylogenetic results do not provide evidence for geneflow between the two subgenera of *Garrya*. It is possible that this lack of geneflow is driven by differences in flowering time, as indicated by a slight tendency toward earlier flowering in subgenus *Garrya* as compared to subgenus *Fadyenia* (Table 1). This idea is supported by the observation of staggered flowering time at several locations in the southwestern U.S. where members of each subgenus occur as part of the same plant communities (D.O. Burge, personal observation).

Acknowledgments

The author thanks the Duke University Systematics Discussion Group for constructive criticism of this work. The author also thanks Katherine Zhukovsky and Kaila Mugford for assistance with lab work. Funding was provided by the Duke University Department of Biology.

Literature Cited

Angiosperm Phylogeny Group, The (APG). 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141:399–436.

Blattner, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. Biotechniques 27:1180–1185.

Bremer, B., K. Bremer, N. Heidari, P. Erixson, R. G. Olmstead, A. A. Anderberg, M. Kallersjo, and E. Barkhordarian. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Molecular Phylogenetics and Evolution 24:274–301.

Dahling, G. V. 1978. Systematics and evolution of *Garrya*. Contributions to the Gray Herbarium of Harvard University 209:1–104.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Felsenstein, J. 1985. Phylogenies and the comparative method. American Naturalist 125:1–15.

Hallock, F. A. 1930. The relationship of *Garrya*. The development of the flowers and seeds of *Garrya* and its bearing on the phylogenetic position of the genus. Annals of Botany 176:771–812.

Liston, A. 2003. A new interpretation of floral morphology in *Garrya* (Garryaceae). Taxon 52:271–276.

Maddison, W. P. and L. L. Knowles. 2006. Inferring phylogeny despite incomplete lineage sorting. Systematic Biology 55:21–30.

Munz, P. A. and D. D. Keck. 1968. A California flora and supplement. University of California Press, Berkeley, CA.

Nylander, J. A. A. 2004. MrModeltest, v2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Soltis, D. E., P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Price, W. J. Kress, K. C. Nixon, and J. S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbc*L, and *atp*B sequences. Botanical Journal of the Linnean Society 133:381–461.

Swofford, D. L. 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.01b10, Sinauer Associates, Sunderland, MA.

Taberlet, P., G. Ludovic, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17:1105–1109.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplifications and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. A. Innis, D. Gelfand, J. J. Sninsky, and T. J. White (eds.), PCR protocols: a guide to methods and applications. San Diego: Academic Press, San Diego, CA.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas, Austin, TX.

Appendix 1

Sampled Individuals of *Garrya* and *Aucuba*

Collector and number followed by description of locality. All specimens deposited in the Duke University Herbarium (DUKE).

Aucuba japonica Thunb.—D.O. Burge 363, City of Chico, 1469 Humboldt Rd, Butte Co., CA. ***Garrya buxifolia*** Gray—D.O. Burge 1160, Swede Creek watershed, roadside on FR 2524 (road to Spalding Mill), Josephine Co., OR. ***G. elliptica*** Douglas ex Lindl.—D.O. Burge 382, Seaside, eastern terminus of Kimball Avenue near Fort Ord Military Reservation, Monterey Co., CA; D.O. Burge 386, Mount Tamalpais, roadside on East Ridgecrest Boulevard, near Middle Peak, Marin Co., CA. ***G. flavescens*** S. Watson —D.O. Burge 370, Ball Mountain, western slope, along Caliente Bodfish Rd, Kern Co., CA; D.O. Burge 419, Wilson Mountain, Wilson Mountain Trail, Yavapai Co., AZ; D.O. Burge 1036, Cerro Bola, eastern slope, Baja California, Mexico. ***G. fremontii*** Torr.—D.O. Burge 353, Doe Mill Ridge (ridge between Butte Creek and Little Chico Creek), Butte Co., CA; D.O. Burge 362, Trinity River canyon, Poison Gulch, Humboldt Co., CA; D.O. Burge 1148, North Fork Tuolumne River watershed, Bald Mountain, Tuolumne Co., CA. ***G. glaberrima*** Wangerin—D.O. Burge 1025, Cerro Juarez, near summit, Hidalgo, Mexico; D.O. Burge 1216, Cerro El Potosí, eastern slope, Nuevo Leon, Mexico; D.O. Burge 1225, Sierra El Pedregoso, Tamaulipas, Mexico. ***G. grisea*** Wiggins—D.O. Burge 778, Sierra San Pedro Mártir, Baja California, Mexico. ***G. laurifolia*** Benth.—D.O. Burge 1218, Cerro El Potosí, eastern slope, Nuevo Leon, Mexico; D.O. Burge 1252, Cascada de Basaseachi area, Chihuahua, Mexico. ***G. lindheimeri*** Torr.—D.O. Burge 750, City of Austin, Mayfield Park and Nature Preserve, Travis Co., TX. ***G. ovata*** Benth.—D.O. Burge 1221, Sierra Los Soldados, Nuevo Leon, Mexico. ***G. veatchii*** Kellogg—D.O. Burge 378, Cuesta Ridge, San Luis Obispo Co., CA; D.O. Burge 1041, Isla Cedros, N slope of Cerro Redondo, Baja California, Mexico. ***G. wrightii*** Torr.—D.O. Burge 934, Santa Catalina Mountains, Pima Co., AZ; D.O. Burge 1239, Sierra de Coneto, western slope, Durango, Mexico; D.O. Burge 1253, Cascada de Basaseachi area, Chihuahua, Mexico.